REMARKS

Applicants have amended previously presented dependent Claim 44 to conform that claim with the other new claim, Claim 44, added in applicant's previously presented amendment. It is emphasized that the amendment to Claim 44 adds no new matter to the application.

In addition, minor grammatical errors present in Claims 16, 19 and 23 have been corrected. These changes, like the amendment to Claim 44, add no new matter or present new issues requiring further search or consideration.

All the claims submitted for examination in this application have been rejected on substantive grounds. Applicants have considered this ground of rejection and respectfully submit that all the claims currently in this application are patentable over this ground of rejection.

The substantive ground of rejection is directed to all the claims currently in this application, Claims 1-44. Claims 1-44 stand rejected, under 35 U.S.C. §103(a), as being unpatentable over U.S. Patent 4,359,430 to Heikkilä et al. in view of Chinese Patent Publication 1234404A to Qiu

The Official Action argues that Heikkilä et al. teaches a process of separating betaine from sugars and nonsugars of beet molasses by a chromatographic process. Heikkilä et al. discloses the use of a column containing a strong cation exchanger in alkali form and the eluting material is generally water at a temperature of 60°C to 90°C. Suffice it to say, Heikkilä et al. does not meet the requirement of every claim of the present application which requires chromatographic separation wherein a weak acid cation exchange resin is used for the chromatographic separation.

To overcome this critical deficiency in the principal Heikkilä et al. reference, a secondary reference, Chinese Patent Publication 1234404A to Qiu, is applied. The English abstract of Qiu, supplied by the PTO, indicates that that patent publication presents a method of separating D-ribose from fermented liquor by the use of an ion-exchange resin wherein the pretreated fermented liquor is passed through a strong-acid cation exchange resin column, a weak-base anion exchange resin column and a weak-acid cation exchange resin column. The Official Action therefore concludes that it would be obvious to one of ordinary skill in the art to use a multi-column chromatographic process with at least one weak-acid cation exchange resin to separate sugars and non-sugars from a solution.

Applicants have previously argued that the use of a weak acid cation ion-exchange resin column, effected by ion exchange separation, is clearly distinguished and different from the claimed chromatographic separation employing a weak acid cation exchange resin. Applicants respectfully maintain their position that chromatographic separation of the type required by all the claims of the present application is clearly patentably distinct from a process involving ion exchange separation as taught by the combined teaching of the applied references.

To support applicants' strongly held position that ion exchange and chromatographic separations are patentably distinct of each other, they enclose a complete translation, rather than the brief English language abstract supplied by the United States Patent and Trademark Office, of the Qiu patent publication. This translation makes it abundantly clear that the separation of D-ribose from fermented liquor occurs by ion-exchange resin separation.

Although not set forth in Qiu, those skilled in the art are aware that ion exchange columns have to be regenerated. For example, the third stage of the Qiu process employs a

weak acid cation exchanger which must be regenerated. That is, the third stage of Qiu requires the column to be loaded with six to eighteen volumes of fermentation broth per volume of resin. After this broth is loaded into and passed through the column, the column has to be regenerated to re-exchange hydrogen for the calcium, magnesium, potassium and sodium cations removed from the broth and now held by the resin so that the column can be recharged with the broth to remove further amounts of these cations.

Similarly, the first stage of the Qiu process, involving passage of the fermented liquor through a strong-acid cation exchange resin, has to be similarly regenerated by passing a strong acid to wash out the cations, e.g. calcium, magnesium, potassium and sodium, that were removed in that step. Likewise, the second stage, the stage wherein a weak-base anion exchange resin removes anions, such as SO_4^{2-}, PO_4^{3-} and Cl^-, must be regenerated by passing a strong base through the column.

The above remarks emphasize that in an ion exchange process, such as that provided by Qiu, each stage of the process results in the absorption of a specific group of ions from a multi-compound fermentation broth. This absorption results in the specific removal of groups of compounds. These specific groups of removed compounds do not travel at different rates through the system. Rather, the ions are fixed to the resin. For example, the cations in the Qiu first column stay there until regenerated. The anions of the second column also remain in that column until regenerated. Similarly, the cations in the third exchanger, which serves in Qiu to remove color from the fermented liquor, has to be regenerated with a strong acid to wash out color absorbed by the resin. The fourth stage, which employs a weak-base anion exchanger to increase pH and thus neutralize the solution as well as remove color, like the third stage, must be regenerated to not only provide base to neutralize the acid solution but

also to wash out the color absorbed by the resin. Upon regeneration, the regenerated resins provide further separation of the fermentation broth.

In summary, the process taught by Qiu comprises absorption of a specific group of compounds from a multicomponent fermentation broth in each stage of a multistage process. These specific groups of compounds do not travel throughout the system at different rates. The only material that flows through the whole of the ion exchange process taught by Qiu is the ribose solution. The ribose solution travels through the four-column system and is removed from the last column by washing with deionized water.

In a chromatographic system, on the other hand, different compounds or group of compounds travel through a resin bed system at different rates. Separated fractions can thus be collected at the outlet of the system due to speed differentials. Each compound or group of compounds is removed from the system at a predictable time due to the knowledge of typical retention time periods for these compounds or group of compounds.

A chromatographic system is also distinguished from an ion exchange system by the absence of any need for regeneration. Thus, anions and cations are not removed from columns with acids and bases after each cycle, as required by ion exchange processes as exemplified by Qiu.

These fundamental principles of ion exchange and chromatographic separations establish the non-equivalence of the weak acid cation exchange resin employed in the Qiu ion exchange process with the weak acid cation exchange resin employed in the claimed chromatographic separation process of the present application.

The ion exchange process disclosed by Qiu, employs a third column, which employs a weak acid cation exchange resin, to remove color from the fermentation broth. On the other

hand, in the chromatographic system of the present application the weak acid cation exchange resin is employed to enhance chromatographic separation of multicompound feed solutions.

It is emphasized that the use of a weak acid cation exchange resin in chromatographic separation is patentably distinct from separation processes of the prior art. This is implicitly conceded in the prosecution of this application by the failure to rely on any teaching of a chromatographic separation employing a weak acid cation exchange resin. Although applicants rely on no theory explaining the operability of the present invention, it is theorized that the claimed weak acid exchange resin changes the order of elution of the different compounds in the multi-compound feed solution.

Prior art multi-compound separations of the prior art employed strong acid ion exchange resins. Strong acid ion exchange resins have different orders of elution. When this fact is combined with the failure of Qiu to disclose, teach or even hint that different compounds or groups of compounds travel through a separation system at different rates in each column, it is apparent that Qiu does not provide the disclosure necessary to supplement the conceded inadequacies of Heikkilä et al. in making obvious any of the claims of the present application.

The above remarks establish that the combined teaching of Heikkilä et al. and Qiu does not make obvious the claims of the present application. Heikkilä et al. discloses a prior art chromatographic process for separating betaine from molasses by using a chromatographic column of a salt of a polystyrene sulfonate cation exchange resin cross-coupled with divinyl benzene and eluted with water. As stated in the specification of the present application at Page 1, the use of water as an eluting agent presents the problem of similar retention times for the various products, e.g. betaine, erythritol, inositol, sucrose, mannitol, amino acids and

mixtures of amino acids, of the present invention whereby the fractions overlap, making separation of these products very difficult. This problem has been solved by the chromatographic separation process of the present application, using a weak acid cation exchange resin.

The Official Action appreciates the deficiency of Heikkilä et al. and thus applies Qiu for its disclosure of a weak acid cation exchanger. However, the Qiu process uses a weak acid cation ion exchange resin which does not produce the results obtained in the claimed chromatographic separation process of the present application. As such, the combined teaching of the two applied references does not only not disclose the claimed chromatographic process employing a weak acid cation exchange resin employed in a chromatographic separation but, in view of the above remarks, does not suggest such a system to one skilled in the art. Therefore, reconsideration and removal of the substantive rejection of record is deemed appropriate. Such action is respectfully urged.

The above amendment and remarks establish the patentable nature of all the claims currently in this application. Notice of Allowance and passage to issue of these claims, Claims 1-45, is therefore respectfully solicited.

Respectfully submitted,

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Chinese patent application title:

Method for separating D-ribose from fermentation liquor

Patent number: CN1234404
Publication date: 1999-11-10
Inventor(s): QIU WEIRAN (CN)
Patent assignee(s): HUADONG UNIV OF SCIENCES AND E (CN)
Wished Patent: CN1234404
Application number: CN19990113547 19990322
Priorities(s): CN19990113547 19990322
IPC Class: C07H1/06; C07H3/02; C12P19/02
EC Classe:
Corresponding application: CN1081191B

SUMMARY

The method for separating D-ribose from fermented liquor by using ion-exchange resin is characterized by that the pre-treated fermented liquor is passed through in turn the strong-acid cation exchange resin column, weak-base anion-exchange resin column and weak-acid cation exchange resin column at flow rate of 1-3 m/hr and the column is washed by deionized water, the effluent is collected, then the conventional method is used to collect D-ribose from the effluent, and its yield can be up to above 95%.

CLAIMS

1. A method to separate D-ribose from fermentation broth (=fermented liquor) is composed of 3 steps: (1) pre-treatment of fermentation broth, (2) purification of the fermentation broth, (3) post-treatment of the purified fermentation broth. Step 2 of the purification of the fermentation broth is carried out in the following manner:

The pre-treated fermentation broth is passed in turn the following ion exchange columns at a linear flow rate of 1-3 meter/hour: Strong acid cation exchanger column (1), weak alkaline anion exchanger column (2), weak acid cation exchanger column (3). After that the columns are washed with de-ionized water, effluent is collected. The optimal ratio between the fermentation broth to strong acid cation exchanger resin and weak alkaline anion exchanger resin are:

Fermentation broth : Resin = (3~9): 1 (v/v)

The optimal ratio of the fermentation broth to weak acid cation exchanger resin is

Fermentation broth : Resin = (6~18): 1 (v/v)

2. As the method described in Claim 1, the characteristic feature of the method is that weak acid cation exchanger column (3) is further connected to weak alkaline anion exchanger resin column (4). The optimal ratio of the fermentation broth to the weak alkaline anion exchanger resin is:

Fermentation broth : Resin = (6~18): 1 (v/v)

3. As the method described in Claim 1-2, the characteristic feature of the method is that: the strong acid cation exchanger in resin column (1) is 732. 001 x 7 strong acid styrene ion exchanger or one of the resins of Amberlite IR-120.

The weak alkaline anion exchanger in column (2) is D-315 large pore size weak alkaline acrylic acid anion exchanger, weak alkaline 330 resin, D301, D309, D396, D351, 709, or one of the resins from Amberlite IRA-63 or IRA-94;

The weak acid cation exchanger resin in column (3) is weak acid resin from the series of HD-1 large pore size phenol-aldehyde resins, one of the resin 122 or 125 series.

The weak alkaline anion exchanger resin in column (4) is D-315 large pore size weak alkaline acrylic acid resin series, weak alkaline resin 330, D301, D309, D396, D351, 709, or one from the Amberlite IRA-63 or IRA-94 series.

INVENTION DESCRIPTION

A method to separate D-ribose from fermentation broth

This invention belongs to the area of biochemical engineering, describing a method to separate D-ribose from fermentation broth, especially using ion exchangers as the tool to perform the separation.

D-ribose is an important constituting component of nucleic acid found in living things. Its derivatives are important components of certain vitamins and coenzymes. It can be the intermediate of Vitamin B_2, it can also be the raw material for various drugs based on nucleosides, and seasonings. D-ribose has therefore a wide application in the area of biochemical engineering.

Before 1980s, D-ribose was mainly made by chemical synthesis abroad. At present, D-ribose production uses the wide spread method of transketolase mutant of the hay *Bacillus subtilis*. From 1990s, fermentation production of D-ribose was started domestically. By fermentation method to produce D-ribose one needs to separate and extract D-ribose from the fermentation mixture. Japanese patent application 56-113297 disclosed a method to separate and extract D-ribose from fermentation broth. At present this method is being used industrially. By this method, the bacterial biomass was first removed from fermentation broth, followed by loading the clear supernatant obtained onto strong acid cation exchanger Amberlite IR-120 (H type) and strong alkaline anion exchanger Amberlite IRA-400 (OH type), to remove the anions and cations from the from fermentation broth, and then concentrated, crystallized to obtain the D-ribose product. By this method to separate and extract D-ribose, the recovery is low due to big loss. When the ribose concentration in the fermentation broth is high, the recovery is 70-80%; when the ribose concentration in the fermentation broth is low, for example, 20-25g/liter, the recovery is 40-50%. The reason is that the hydroxyl groups of the ribose have an isoelectric point of pH 12. At pH 12, there are quite a lot of hydroxyl groups that undergo de-protonation, resulting negative charges, which can ion exchange with strong alkaline anion exchanger. It is then difficult to elute the ribose from this exchanger using water, resulting low D-ribose recovery. Besides, the purity of ribose was

worsened by the presence of large amount of pigments from the fermentation broth. Activated carbon can be used for de-colouring, but it is not a good method and final product is brownish syrup. Therefore it is necessary to develop a new method for separating and extracting ribose from fermentation broth to meet the needs of production sectors.

The goal of the current invention is to disclose a new method to separate ribose from fermentation broth. The method is based on using weak alkaline anion exchanger instead of strong alkaline anion exchanger that is used currently. This method is further complemented with weak acid cation exchanger. This makes it possible to have a composite optimal system having high efficacy of removing impurities, low loss, for use in the purification of D-ribose fermentation broth. This therefore overcomes problems of the today-used technique

The reasoning and thought behind the current invention:
The solution is acidic when fermentation broth is passed through a strong acid cation exchanger, while weak alkaline anion exchanger has a pH exchanging range of pH 0-8, generally inorganic anions could have ion exchange and be adsorbed, whereas D-ribose has a dissociation at pH 12, and can not be adsorbed. In this way inorganic anions were removed form fermentation broth, such as SO_4^{2-}, $PO4_4^{3-}$, and Cl^- etc. This can also avoid the adsorption of D-ribose, resulting in loss and low recovery. This weak alkaline anion exchanger can also adsorb pigments from the fermentation broth, therefore increasing the purity of D-ribose.

The current invention is realized in the following manner:

The method of the current invention consists of 3 parts:

(1) Pre-treatment of the fermentation broth
(2) Purification of the fermentation broth
(3) Post-treatment of the purified liquor

Detailed process is as follows:

(1) Pre-treatment of the fermentation broth: Bacterial biomass and solid impurities from the fermentation broth or from the fermentation broth with coagulant added are removed by centrifugation with centrifuge or filtering and pressured filter device. There is no need for detailing it here as there are so many literatures on this subject.

(2) Purification of the fermentation broth

1---------- Strong acid cation exchanger column
2---------- Weak alkaline anion exchanger column
3---------- Weak acid cation exchanger column
4---------- Weak alkaline anion exchanger column

The fermentation both has a D-ribose content of about 40g/liter after the removal of bacterial biomass and solid matter. It has cations of calcium, magnesium, potassium and sodium and anions of sulfate, phosphate, and chloride etc. The solution is in brown colour. Load this liquor in a linear flow rate of 1-3 meter/hours in the following order on strong acid cation exchanger column (1), weak alkaline anion exchanger column (2), weak acid cation exchanger column (3). Wash the columns with de-ionized water to wash out the D-ribose adsorbed onto the column. In this way a

clear liquor of D-ribose free of cations and anions is obtained. The role of strong acid cation exchanger column (1) is to remove cations of calcium magnesium, potassium and sodium etc. The role of weak alkaline anion exchanger column (2) is to remove anions of sulfate, phosphate and chloride etc., as well as most of the pigments. The role of weak acid cation exchanger column (3) is to remove the residual pigments in the liquor. The obtained liquor is acidic and has a pH of 3-5. The content of D-ribose is about 30g/liter. In order to obtain neural liquor and to remove residual pigments, weak alkaline anion exchanger resin column is further used to obtain high purity D-ribose product.

The strong acid cation exchanger in resin column (1) is 732. 001 x 7 strong acid styrene ion exchanger or one of the resins of Amberlite IR-120 series.

The weak alkaline anion exchanger in column (2) is D-315 large pore size weak alkaline acrylic acid anion exchanger, weak alkaline 330 resin, D301, D309, D396, D351, 709, or one of the resins from Amberlite IRA-63 or IRA-94 series. There is a special ratio between the amount resin used and the volume of fermentation broth. The optimal ratio between the fermentation broth and strong acid cation exchanger resin and weak alkaline anion exchanger resin are:

Fermentation broth : Resin = (3~9): 1 (v/v)

The weak acid cation exchanger resin in column (3) is weak acid resin from the series of HD-1 large pore size phenol-aldehyde resins, one of the resins of 122 or 125 series. The optimal ratio is:

Fermentation broth : Resin = (6~18): 1 (v/v)

The weak alkaline anion exchanger resin in column (4) is D-315 large pore size weak alkaline acrylic acid resin series, weak alkaline resin 330, D301, D309, D396, D351, 709, or one from the Amberlite IRA-63 or IRA-94 series. The optimal ratio is:

Fermentation broth : Resin = (6~18): 1 (v/v)

(3) Post-treatment of the purified liquor:

The clear liquor obtained from Step 2 having D-ribose can be, by conventional separation method, concentrated, crystallized to get pure D-ribose product.

By adopting the method described in this invention, the procedure is simple, easy to operate, and high recovery of D-ribose of 95%. The product D-ribose has high purity. Therefore it is a good separation method that bears the future for industrial application.

Below the invention detail and examples are given:

Example 1

Centrifuge the fermentation broth at 8000 rpm for 30 min to remove the bacterial biomass. D-ribose content was 36g/liter. Pass this broth at 1.5 meter/hour linear flow rate through columns packed with 732 resin, D-315 resin and HD-1 resins. The amount of resin 732 was 300 ml, column was Φ26x800mm. The resin amount for D315 was 300 ml, column was Φ26x800mm. The resin

amount for HD-1 was 150 ml, column was Φ26x600mm. After loading, wash the columns with 1000 ml de-ionized water. The effluent from the columns was colourless and transparent, no Cl^- was detected by $AgNO_3$ method. 1200 ml effluent containing D-ribose was collected. D-ribose content was 28.8g/liter, pH was 5. Recovery was 96 (wt%). After concentrating the effluent, crystallization and vacuum-drying, the final product of D-ribose had a purity of 98%.

Example 2

Centrifuge and filter the fermentation broth to remove the bacterial biomass. D-ribose content was 40g/liter. Pass this broth 40 liter at 2 meter/hour linear flow rate through 4 resin columns packed with 732 resin, D-315 resin, HD-1 resin and D-315 resin. The amount of resin 732 was 10 liter, the column was Φ150x1000mm. The resin amount for D315 was 10 liter, column was Φ150x100mm. The resin amount for HD-1 was 5 liter, column was Φ100x1000mm. The fourth column had 5 liter D315 resin, column dimension was Φ100x1000mm After loading the broth, wash the columns with 50 liter de-ionized water. The effluent of 65 liter collected from the columns was colourless and transparent. D-ribose content was 22.6g/liter, the pH was 7. Recovery was 92 (wt%). After concentrating the effluent, crystallization and vacuum-drying, the final product of D-ribose had a purity of 98%.

Example 3

Centrifuge the fermentation broth to remove the bacterial biomass. D-ribose content was 36g/liter. Pass this broth 1000 ml at 1.5 meter/hour linear flow rate through columns packed with 732 resin, Amberlite IRA-63 resin, and 122 resin. The amount of resin 732 was 300 ml, column was Φ26x800mm. The resin amount for Amberlite IRA-63 was 300 ml, column was Φ26x800mm, The resin amount for HD-1 was 150 ml, column is Φ26x600mm. After loading the broth, wash the columns with 1000 ml de-ionized water. The effluent 1200 ml containing D-ribose was collected. D-ribose content was 28.2g/liter, pH was 4. Recovery was 94 % (wt%). After concentrating the effluent, crystallization and vacuum-drying, the final product of D-ribose had a purity of 95%.

Invention description figure:

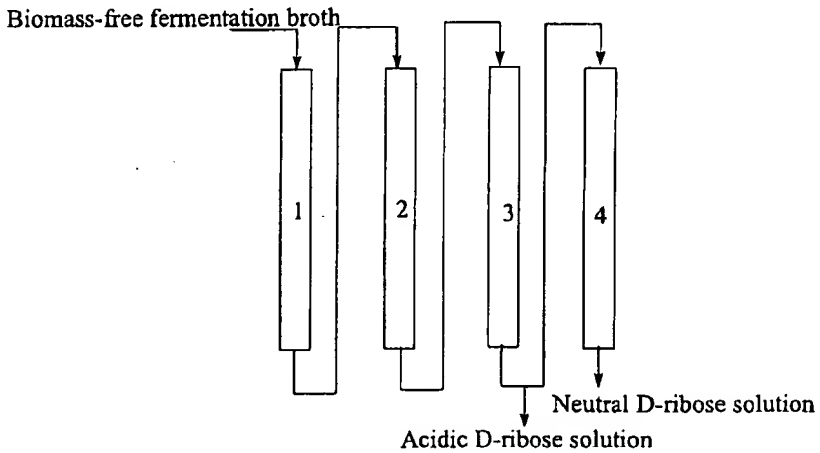

Figure 1